SUPPLEMENTAL MATERIAL

Tusche et al., http://www.jem.org/cgi/content/full/jem.20091802/DC1

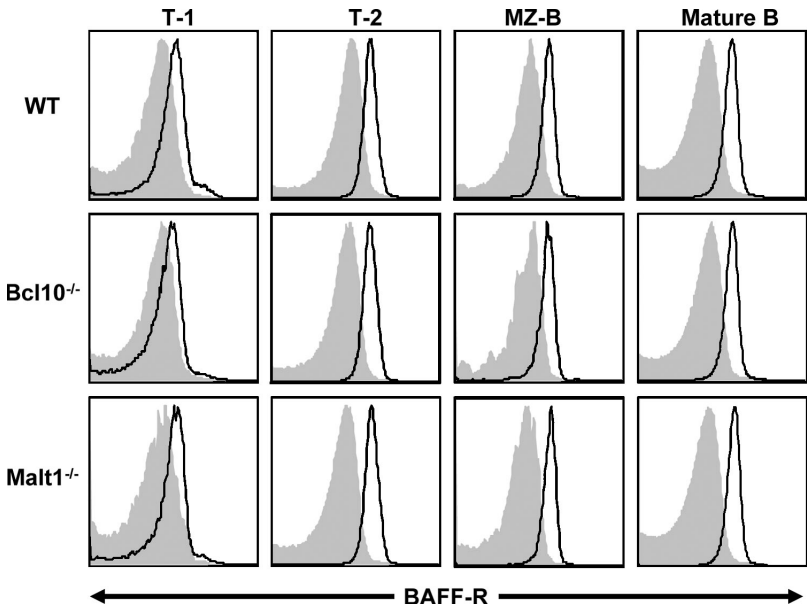

Figure S1. BAFF-R expression. The level of BAFF-R expression on various B cell subsets was determined using flow cytometry. B cells ($B220^+$) in the spleen were separated based on IgM, CD21, and CD23 expression to delineate T1 (IgM^{high}, $CD21^{low}$, $CD23^{neg}$), T2 (IgM^{high}, $CD21^{high}$, $CD23^+$), MZ (IgM^{high}, $CD21^{high}$, $CD23^-$), and mature B cells. Shaded histograms indicate isotype control. The histograms represent similar results obtained from four mice of each indicated genotype.

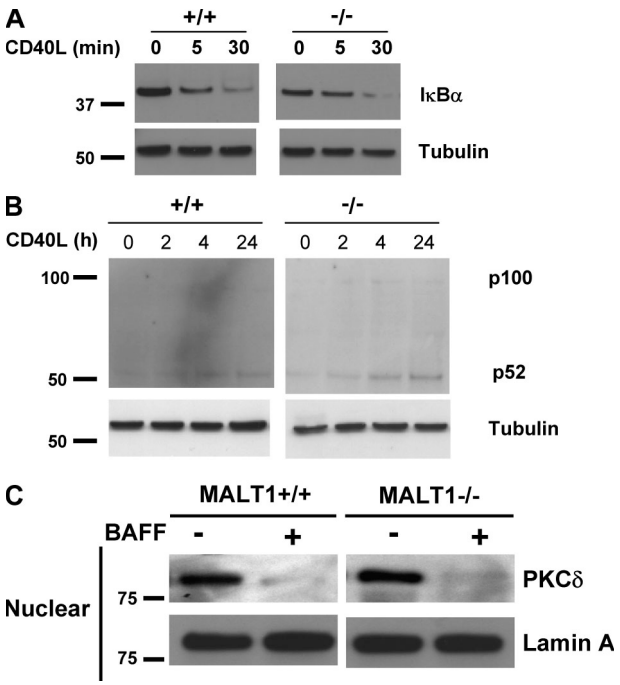

Figure S2. CD40L–induced canonical and noncanonical NF-κB does not require MALT1. B220+ B cells from the indicated genotypes were isolated using negative selection. (A and B) Cells were stimulated with 250 ng/ml CD40L for the indicated times and lysed directly to obtain cytoplasmic extracts. (C) Extracts were Western blotted according to standard protocols and probed for the indicated factors. Results displayed are representative of three independent analyses. Numbers to the left of gels represent kilodaltons.

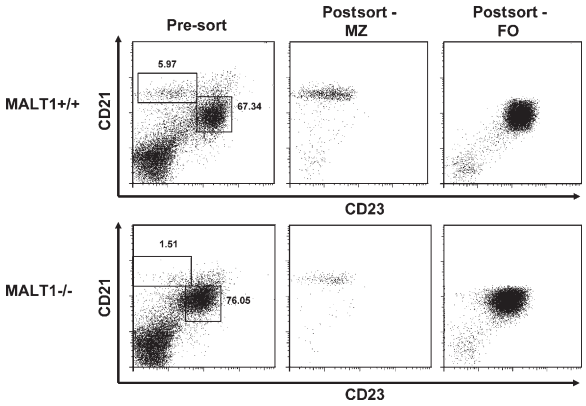

Figure S3. FACS sorting of MZ and FO B cells. B cells from the indicated genotypes were sorted using a FACSAria as described in Materials and methods (percentages are shown). Postsort analysis consistently showed a purity of >99% for FO and MZ B cells.

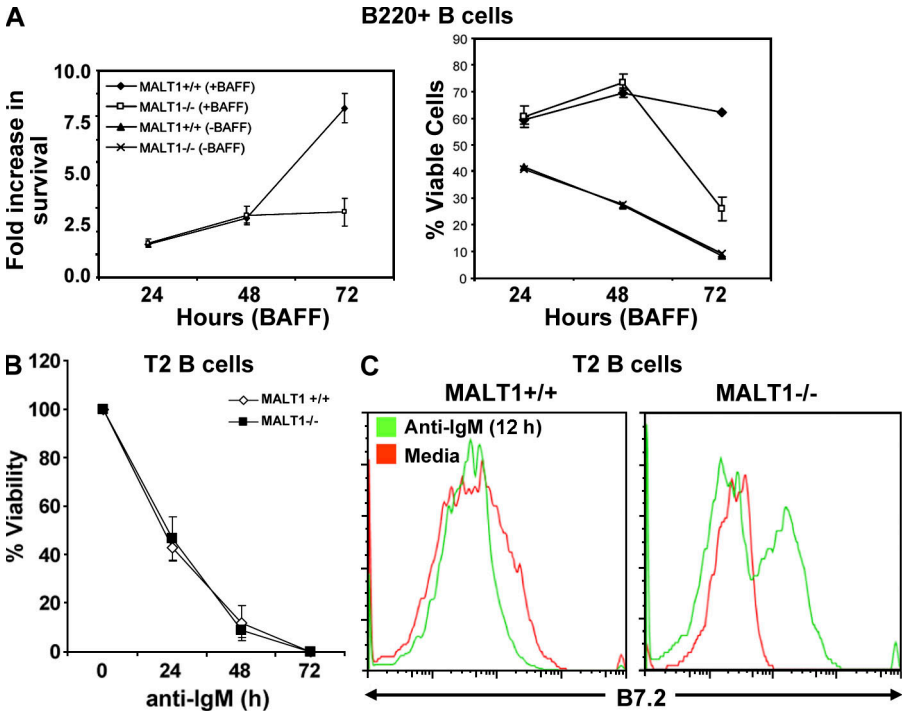

Figure S4. MALT1$^{-/-}$ B220^{+} B cells have reduced survival capacity in response to BAFF while MALT1$^{-/-}$ T2 B cells exhibit normal anti-IgM–induced survival and B7.2 up-regulation. (A) B220^{+} B cells from MALT1$^{+/+}$ and MALT1$^{-/-}$ mice were cultured for the indicated times in medium alone or medium supplemented with 2 µg/ml BAFF. Viability was assessed using annexin V/PI staining. Data are expressed as the fold increase over unstimulated controls (left) or the percentage of viable cells (right). Results shown are means ± SD of one experiment and are representative of three independent analyses. (B) T2 B cells were sorted from MALT1$^{+/+}$ and MALT1$^{-/-}$ mice. T2 cells were treated with 5 µg/ml anti-IgM for the indicated times, and viability was assessed using annexin V/PI staining as in A. (C) B7.2 up-regulation in T2 B cells isolated from the indicated genotypes was measured using flow cytometry after 12 h of anti-IgM (5 µg/ml) or media treatment. Results are representative of two independent analyses.

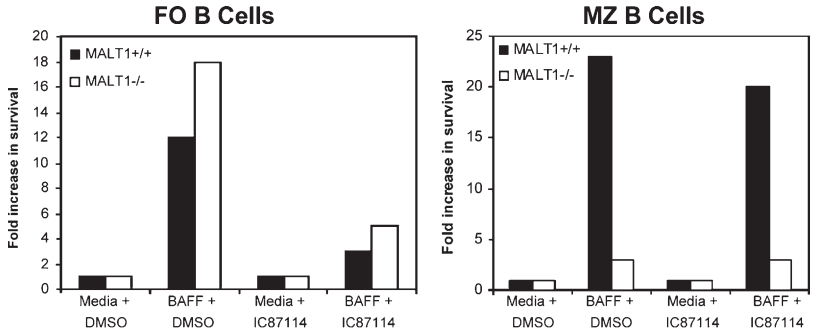

Figure S5. PI3-kinase inhibition abrogates BAFF-induced survival of FO but not MZ B cells. FO and MZ B cells from the indicated genotypes were pretreated with 15 µM IC87114 or DMSO and incubated with either media or 2 µg/ml BAFF for 48 h. Survival is expressed as the fold increase in surviving cells as compared with nonstimulated controls. Data are representative of two independent analyses.

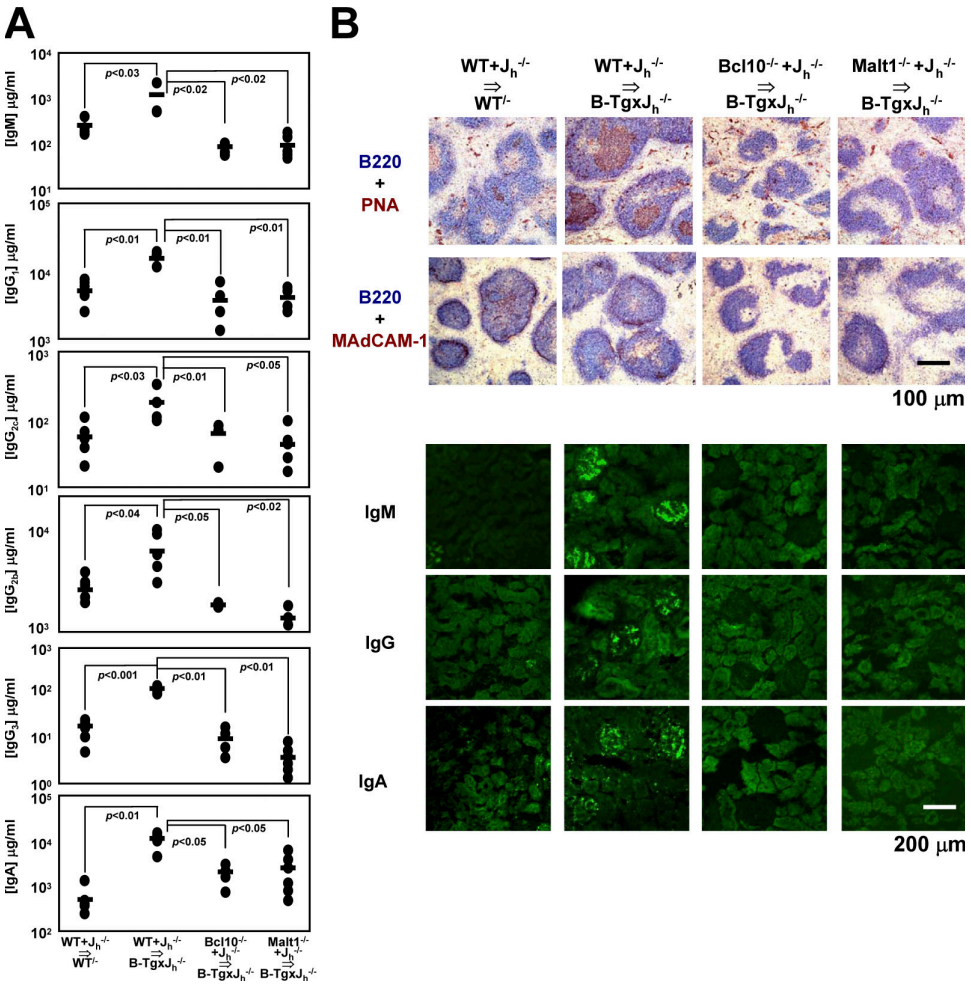

Figure S6. BAFF-induced increases in serum Ig, spontaneous GC formation, and Ig deposition in the kidney require B cell–intrinsic expression of MALT1. Mixed bone marrow chimeras were generated by mixing (1:1) bone marrow derived from the indicated genotypes and injecting it into a lethally irradiated WT or BAFF-Tg × $J_h^{-/-}$ recipient. (A) Serum Ig in the indicated chimeric mice was determined using sandwich ELISA. WT+$J_h^{-/-}$→WT denotes WT bone marrow mixed with $J_h^{-/-}$ bone marrow, introduced into a WT lethally irradiated recipient. Horizontal bars are means. (B) Immunohistochemistry of GCs and immunofluorescence of Ig deposition in the kidney was conducted as described in Fig. 7 (n = 4 mice/genotype). Bone marrow chimeras were examined twice with similar results.

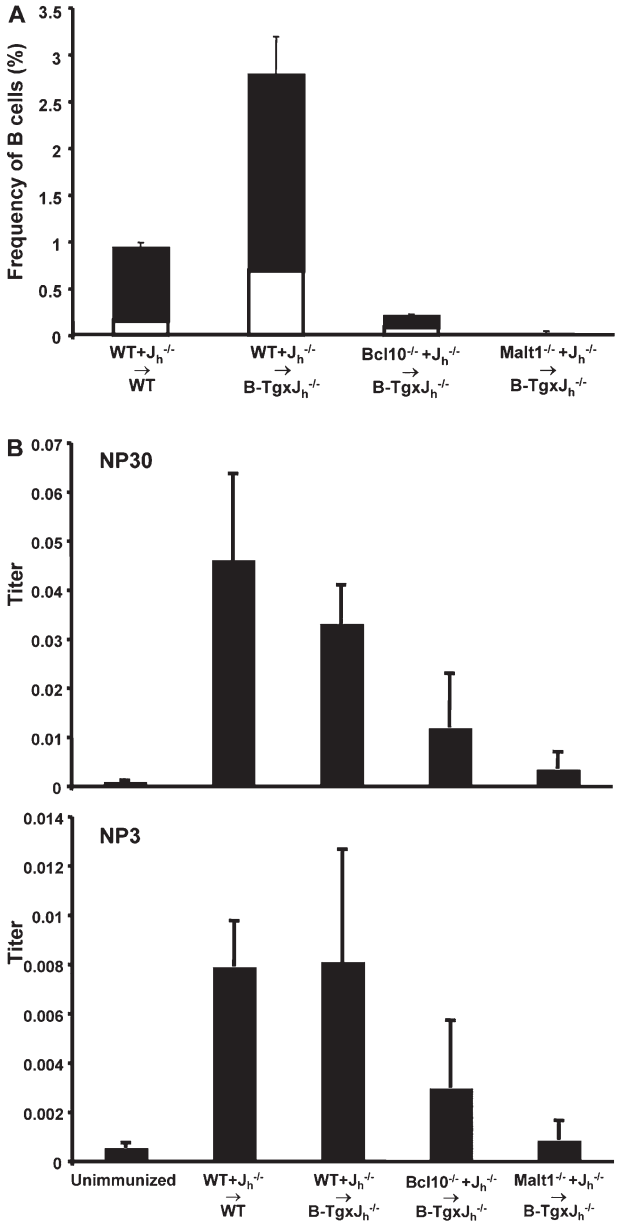

Figure S7. GC formation and antibody response to a Td antigen is dependent on B cell–intrinsic expression of MALT1. (A) The indicated bone marrow chimeric mice (n = 4 mice/genotype) were left unimmunized (white bars) or immunized (black bars) with the Td antigen NP-CGG. The percentage of GC B cells in the spleen was determined using immunostaining followed by flow cytometric analysis. (B) Serum samples from unimmunized and NP-CGG–immunized mice were analyzed using sandwich ELISA for the presence of low affinity (NP-30) and high affinity (NP-3) anti-NP antibodies. Results shown are means ± SD, and data presented are representative of three independent experiments (n = 4 mice/genotype).

Table S1. Absolute cell numbers

Cells ×10⁶		WT	B-Tg	Bcl10$^{-/-}$	MALT1$^{-/-}$	Bcl10$^{-/-}$ × B-Tg	MALT1$^{-/-}$ × B-Tg
Spleen	Total cells	179.5 ± 45.7	322.1 ± 39.8	152.66 ± 13.4	150.1 ±14.9	158.4 ± 53.4	178.1 ± 39
	Total MZB	1.24 ± 0.35	6. ± 1.04	0.29 ± 0.04	0.15 ± 0.02	0.57 ± 0.58	0.47 ± 0.08
	Total T1	3.79 ± 0.83	6.24 ± 1.01	0.4 ± 0.27	3 ± 0.49	3.64 ± 0.98	5.23 ± 0.81
	Total T2	2.67 ± 1.25	6.91 ± 2.18	0.83 ± 1.37	0.84 ± 0.14	5.41 ± 5.19	1.55 ± 0.28
	Total FO	46.24 ± 12.3	82.85 ± 13.89	14.37 ± 6.79	36.74 ± 0.41	17.52 ± 15.41	51.06 ± 12.73
PEC	Total cells	3.24 ± 0.712	4.7 ± 0.7	2.87 ± 1.32	2.21 ± 1.33	2.49 ± 1.05	2.21 ± 1.33
	Total B1 B cells	0.291 ± 0.079	0.639 ± 0.096	0.054 ± 0.027	0.106 ± 0.019	0.039 ± 0.018	0.106 ± 0.019

Peritoneal lavages and single-cell suspensions of splenocytes were obtained from the indicated genotypes. Red blood cells were lysed and cells were counted. Absolute numbers of the indicated B cell populations were calculated based on the percentage of these cells obtained through flow cytometric analyses. At least four mice of each genotype were used to calculate absolute numbers for each population. PEC, peritoneal exudate cells.

Table S2. Oligonucleotides used in real-time PCR analysis

Factor	Sense (5′ to 3′)	Antisense (5′ to 3′)
Bcl-2	ATGCCTTTGTGGAACTATATGGC	GGTATGCACCCAGAGTGATGC
Bcl-xL	AGGCGATGAGTTTGAACTGC	AAAGCTCTGATACGCGGTCC
Cyclin D1	GCGTACCCTGACACCAATCTC	CTCCTCTTCGCACTTCTGCTC
Cyclin D2	GAGTGGGAACTGGTAGTGTTG	CGCACAGAGCGATGAAGGT